

Figure S1. Average value of split amino acid composition in mitochondrial (mp) and nonmitochondrial (non-mp) proteins. 30 NT indicates the first 30 N-terminal residues, and exc30NT indicates the remaining residues excluding the 30NT.



Figure S2. The fluorescence microscopy images of two proteins with potential endoplasmatic reticulum (ER) localization. The first column depicts the protein of interest tagged to GFP, the second column shows the Mitotracker staining, and the third column represents the overlay including DAPI and differential interference contrast (DIC) image. Both proteins were predicted to localize to the mitochondrion, but clearly showed non-mitochondrial localization in procyclic *T. brucei* cells when tagged with GFP.



FigureS3. RNAi knockdown of the mitochondrial Succinyl CoA synthetase beta subunit in (A, B) bloodstream and (C, D) procyclic form T. brucei. The graphs (A, C) show accumulative cell growth with (red) and without (blue) addition of tetracycline (1 ug/ml). For the northern hybridization (B, D), RNA was prepared after 18 hours post induction and 10ug of RNA were loaded in each lane and probed for the Succinyl CoA synthetaset by a <sup>32</sup>P labeled oligonucleotid.